EFSA Panel on Dietetic Products, Nutrition and Allergi es (NDA) ; Scientific Opinion - Statement on the safety of the “conjugated linoleic acid (CLA) - rich oils” Clarinol ® and Tonalin TG 80 as Novel Food ingredients

EFSA Publication

Link to article, DOI:
10.2903/j.efsa.2012.2700

Publication date:
2012

Document Version
Publisher's PDF, also known as Version of record

SCIENTIFIC OPINION

Statement on the safety of the "conjugated linoleic acid (CLA)-rich oils" Clarinol® and Tonalin® TG 80 as Novel Food ingredients[1]

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to update its opinions on the safety of the conjugated linoleic acid (CLA)-rich oils Clarinol® and Tonalin® TG 80 as Novel Food ingredients in the light of additional information provided by Member States to the European Commission. Clarinol® and Tonalin® TG 80 consist of approximately 80 % of the two CLA isomers *c-9,t-11* and *t-10,c-12* (1:1). The applicants suggested a daily intake of CLA of 3 g (3.75 g Clarinol®) and 3.5 g (4.5 g Tonalin® TG 80), respectively. The Panel considers that the additional information provided does not contain evidence thath would modify its previous conclusions regarding the effects of CLA on insulin sensitivity/glucose metabolism, blood lipids, lipid peroxidation, or subclinical inflammation. The Panel also considers that the new studies provided do not address longer-term (> 6 months) effects of CLA intake on insulin sensitivity, the arterial wall or liver steatosis, or the safety of CLA in type-2 diabetic subjects, under the proposed conditions of use. The Panel concludes that the safety of Clarinol® and Tonalin® TG 80 has been established for the proposed uses and daily doses for up to six months. The safety of CLA consumption for periods longer than six months has not been established under the proposed conditions of use. The safety of CLA consumption by type-2 diabetic subjects has not been established.

KEY WORDS

Conjugated Linoleic Acid, insulin sensitivity, blood lipids, novel food ingredient, Cognis, Lipid Nutrition

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[1] On request from the European Commission, Question No (EFSA-Q-2012-00300), adopted on 27 April 2012.

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[3] Acknowledgement: The Panel wishes to thank the members of a drafting group of the NDA Panel Albert Flynn, Ambroise Martin, Bevan Moseley, and Hildegard Przyrembel, and EFSA's staff member Silvia Valtueña Martínez, for the preparatory work provided to this EFSA scientific output.

Suggested citation: EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion - Statement on the safety of the "conjugated linoleic acid (CLA)-rich oils" Clarinol® and Tonalin® TG 80 as Novel Food ingredients. EFSA Journal 2012;10(5):2700. [15 pp.]. doi:10.2903/j.efsa.2012.2700. Available online: www.efsa.europa.eu/efsajournal

SUMMARY

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to update its opinions on the safety of the conjugated linoleic acid (CLA)-rich oils Clarinol® and Tonalin® TG 80 as Novel Food ingredients in the light of additional information provided by Member States to the European Commission.

Clarinol® and Tonalin® TG 80 consist of approximately 80 % of the two CLA isomers *c-9,t-11* and *t-10,c-12* (1:1). Clarinol® and Tonalin® TG 80 were intended by the applicants as ingredients in beverages, cereal products, dietary supplements, and milk products for adult consumers. The applicants suggested a daily intake of 3 g CLA, corresponding to approximately 3.75 g Clarinol®, and of 3.5 g, corresponding to approximately 4.5 g Tonalin® TG 80, respectively.

In its previous opinions, the Panel considered that CLA consumption did not appear to have adverse effects on insulin sensitivity, blood glucose control or liver function for periods up to six months, and that the observed effects on blood lipids were unlikely to have an impact on cardiovascular disease risk. However, the observed increase in plasma and urinary concentrations of isoprostanes, which may indicate an increase in lipid peroxidation, and the increase in some markers of subclinical inflammation (i.e., 15-*keto*-dihydroprostaglandin $F_{2\alpha}$ and possibly CRP) associated with CLA consumption, together with the limited data available on the effects of CLA on vascular function, may indicate a potential for vascular damage (i.e., atherosclerosis) in the long term. Long-term effects of CLA intake on insulin sensitivity, the arterial wall or liver steatosis had not been adequately addressed in humans. The evidence provided did not establish the safety of CLA consumption by type-2 diabetic subjects under the proposed conditions of use.

The Panel considers that the additional information provided does not contain evidence that would modify the previous conclusions reached by the Panel regarding the effects of CLA on insulin sensitivity, blood glucose control, blood lipids, lipid peroxidation, or subclinical inflammation. The Panel also considers that the new studies provided do not address longer-term (> 6 months) effects of CLA intake on insulin sensitivity, the arterial wall or liver steatosis, or the safety of CLA in type-2 diabetic subjects, under the proposed conditions of use.

The Panel concludes that the safety of Clarinol® and Tonalin® TG 80 has been established for the proposed uses and daily doses (3.75 g Clarinol® and 4.5 g Tonalin® TG 80 corresponding to approximately 3 and 3.5 g of CLA, respectively) for up to six months. The safety of CLA consumption for periods longer than six months has not been established under the proposed conditions of use. The safety of CLA consumption by type-2 diabetic subjects has not been established.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

On 30 April 2010, EFSA adopted two Scientific Opinions on the safety of "conjugated linoleic acid (CLA)-rich oil" as a novel food ingredient [4,5].

The conclusion of the EFSA opinions was that the safety of CLA consumption for more than six months has not been established. Subsequently member States could not support a Commission Decision authorising the use of CLA as a novel food ingredient. Instead member States provided additional information that was considered to be relevant for the safety assessment.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In view of the above the Commission requests EFSA to review and update its opinions in the light of the following additional information:

1. Effect of animal and industrial trans fatty acids on HDL and LDL cholesterol levels in humans-a quantitative review. Browers et al. (2010) PLoS ONE, March 2010, Volume 5, Issue 3, e9434.

2. Effect of a high intake of conjugated linoleic acid on lipoprotein levels in healthy human subjects. Wanders et al (2010) PLoS ONE, February 2010, Volume 5, Issue 2, e9000.

3. Application A1005. Exclusive use of Tonalin® CLA as a novel food assessment report, 13 May 2011, Food Standards Australia and New Zealand (FSANZ).

4. Publication of the Superior Health Council No. 8736. Novel food ingredients: oils rich in conjugated linoleic acid in food, 6 July 2011.

5. Opinion of the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) on a "safety assessment of the use of an oil enriched with Conjugated Linoleic Acid (CLA)". 7 October 2011. Request no. 2011-SA-0185

[4] EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the safety of "conjugated linoleic acid (CLA)-rich oil" (Cognis) as a Novel Food ingredient. EFSA Journal 2010; 8(5):1600 [43 pp.]. doi:10.2903/j.efsa.2010.1600.

[5] EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the safety of "conjugated linoleic acid (CLA)-rich oil" (Lipid Nutrition) as a Novel Food ingredient. EFSA Journal 2010; 8(5):1601 [41 pp.]. doi:10.2903/j.efsa.2010.1601.

EVALUATION

1. Introduction

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to carry out the additional assessment for Clarinol® and Tonalin® TG 80, two conjugated linoleic acid (CLA)-rich oils, as novel food ingredients in the context of Regulation (EC) No. 258/97. Clarinol® and Tonalin® TG 80 consist of approximately 80 % of the two CLA isomers *c-9,t-11* and *t-10,c-12* (1:1). Clarinol® and Tonalin® TG 80 were intended by the applicants as ingredients in beverages, cereal products, dietary supplements, and milk products for adult consumers. The applicants suggested a daily intake of 3 g CLA, corresponding to approximately 3.75 g Clarinol®, and of 3.5 g, corresponding to approximately 4.5 g Tonalin® TG 80, respectively.

The Panel concluded that the safety of Clarinol® and Tonalin® TG 80 had been established for the proposed uses and levels of intake for up to six months, that the safety of CLA consumption for periods longer than six months had not been established under the proposed conditions of use, and that the safety of CLA consumption by type-2 diabetic subjects had not been established.

These conclusions were based on the following considerations:

1. The administration of CLA to normal weight, overweight and obese non-diabetic subjects did not appear to have adverse effects on insulin sensitivity, blood glucose control or liver function at the proposed conditions of use for up to six months.

2. The effects of CLA consumption over periods longer than six months on insulin sensitivity and liver steatosis had not been adequately addressed in humans.

3. CLA appeared to adversely affect both static and dynamic surrogate markers of insulin sensitivity as well as fasting blood glucose concentrations in subjects with type-2 diabetes and no studies on blood glucose control (e.g., HbA1c) were available for periods of consumption beyond eight weeks in this population subgroup.

4. Under the proposed conditions of use, CLA was considered to have no effect on LDL-cholesterol concentrations or the LDL:HDL-cholesterol ratio, and the magnitude of the changes observed in HDL- and triglyceride concentrations were unlikely to have an impact on CVD risk.

5. The observed increase in plasma and urinary concentrations of isoprostanes, which may indicate an increase in lipid peroxidation, and the increase in some markers of subclinical inflammation (i.e., 15-*keto*-dihydroprostaglandin $F_{2\alpha}$ and possibly CRP) associated with CLA consumption, together with the limited data available on the effects of CLA on vascular function, were considered to indicate a potential for vascular damage (i.e., atherosclerosis) in the longer term, and no data on effects of CLA intake on the arterial wall had been provided in humans.

2. Additional information provided

In the context of the present opinion, the three reports from National Authorities provided in the Terms of Reference (ToR) will be considered as sources of information that may not have been taken into account in previous evaluations (EFSA 2010a and b). In this respect, *in vitro* and animal studies showing an effect of CLA isomers, and in particular of the *t-10,c-12* CLA isomer, on glucose uptake, insulin sensitivity or blood glucose control were considered to identify critical outcomes in relation to glucose homeostasis for the safety assessment of CLA isomers in human intervention studies, which were the basis for the conclusions of the NDA Panel. The Panel considers that recently published *in*

vitro and animal studies that have assessed the effects of CLA on e.g., measures of glucose uptake or insulin sensitivity (Halade et al., 2010; Hommelberg et al., 2010; Kennedy et al., 2009; 2010a) do not provide additional information about the safety of CLA consumption in humans.

3. Effects of CLA consumption in humans

Among the additional human studies identified in the opinion by the French Agency for Food, Environmental and Occupational Health and Safety (ANSES, 2011) as not being considered by EFSA (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2010a, 2010b), the Panel considers that only human intervention studies testing the effects of CLA *vs*. a control fat may allow conclusions to be drawn on the safety of CLA. Therefore, case control studies reporting on CLA content in adipose tissue rather than on CLA intakes (Smit et al., 2010), one-arm studies without a control group (Thrush et al., 2007), studies using dairy products high or low in ruminant-derived mixes containing *trans*-vaccenic acid and the *c-9,t-11* isomer of CLA (Brown et al., 2011; Desroches et al., 2005; Sofi et al., 2010; Tricon et al., 2006), studies giving CLA in milk or yoghurt drinks without replacing CLA with fat in the placebo vehicle (Bonet Serra et al., 2008), and studies in which CLA was given in conjunction with other potentially active ingredients without a CLA arm only for comparison (Ahren et al., 2009), have not been considered as sources of data for this evaluation.

3.1. Effects on insulin sensitivity and glucose metabolism

Since publication of the EFSA opinions, four additional randomised controlled trials (RCTs) in humans have been reported on the effects of CLA on fasting blood glucose and insulin concentrations and on the static surrogate marker of insulin resistance HOMA-IR (Joseph et al., 2011; Pfeuffer et al., 2011; Racine et al., 2010; Sluijs et al., 2010). None of these studies had insulin sensitivity or glucose homeostasis as a primary outcome, but rather body weight and body composition (Joseph et al., 2011; Racine et al., 2010), aortic pulse wave velocity (PWV, Sluijs et al., 2010), or endothelial function (Pfeuffer et al., 2011).

In the study by Racine et al. (2010) 62 overweight or obese pre-pubertal children aged 6-10 years were randomised to consume 2.4 g/day of CLA (50:50 mixture of the *c-9, t-11* and *t10,c-12* isomers) or a control fat (sunflower oil) in milk chocolate for 6 months. The study by Sluijs et al. (2010) was conducted in 401 overweight or obese adults (342 analysed) aged 40–70 years, randomised to receive 3.1 g/day CLA (80:20 mixture of the *c-9, t-11* and *t10,c-12* isomers) or a control fat (a blend of 80 % palm oil and 20 % soybean oil) in capsules for 6 months. In the study by Pfeuffer et al. (2011), 85 overweight men aged 45–68 years were randomized to receive 4.5 g/day of the 50:50 CLA isomeric mixture (3.6 g/day CLA), safflower oil, heated safflower oil, or olive oil for four weeks. The study by Joseph et al. (2011) was a 3-phase crossover trial conducted in 27 overweight males aged 18-60 years. Participants consumed in random order 3.5 g/day of safflower oil (control), of a 50:50 CLA isomeric mixture (2.8 g/day of CLA) and the CLA *c-9, t-11* isomer (2.7 g/day CLA) for eight weeks each with a 4-week washout in between. No significant differences between the CLA and control groups on changes in fasting blood glucose or insulin concentrations, or in the HOMA-IR when calculated (Joseph et al., 2011; Pfeuffer et al., 2011; Sluijs et al., 2010), were reported in these studies. With the exception of the Pfeuffer et al. (2011) study, where body weight was reported to significantly decrease in the CLA group compared to all three control fats, body weight changes did not differ between the CLA and control groups.

In the study by Pfeuffer et al. (2011), the glucose and insulin areas under the curve calculated from values at different time points for five hours after consumption of a standardised meal did not differ significantly between the CLA and control groups.

The Panel notes that all these studies administered CLA for ≤ 6 months, were conducted in overweight or obese (and presumably non-diabetic) subjects, did not report on adequate measures of

insulin sensitivity or blood glucose control and were not powered on this basis. The Panel also notes that no new studies are available in type-2 diabetic subjects.

The Panel considers that the additional information provided does not contain evidence that would modify the previous conclusions reached by the Panel regarding the effects of CLA on insulin sensitivity and glucose metabolism.

3.2. Effects on blood lipids and lipoproteins

Previous conclusions on the effects of CLA on the blood lipid profile (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2010a, 2010b) were based on two meta-analyses provided by the applicant (Clifton, 2009; Herrmann, 2009), which already considered the results from the study by Wanders et al., (2010a) cited in the ToR as additional information. In these meta-analyses, no significant effect of CLA consumption was observed on LDL-cholesterol concentrations or the LDL:HDL-cholesterol ratio compared to control fats. Blood HDL-cholesterol concentrations were reduced significantly in the CLA intervention group (by 5-6 %) compared to placebo (usually olive oil or safflower oil rich in oleic acid) in the meta-analysis by Clifton (2009) and in the meta-analysis by Hermann (2009) when only the eight studies using olive oil as placebo were considered. Blood concentrations of triglycerides were also significantly higher in the CLA group compared to placebo in the overall population, in subjects with a BMI >27 kg/m^2, and in the studies using olive oil as placebo in this meta-analysis (Herrmann, 2009). The Panel noted that a significant (although modest) HDL-cholesterol lowering effect and a significant (although modest) triglyceride raising effect of the 50:50 CLA isomeric mixture could not be excluded, which supported the notion that long-term changes in insulin sensitivity and associated changes in blood lipids may be associated with the chronic administration of CLA. The Panel considered that the magnitude of the changes observed in HDL- and triglyceride concentrations was unlikely to have an impact on coronary heart disease risk.

In the assessment report by the Food Standards Australia and New Zealand (FSANZ, 2011) on the use of Tonalin® CLA as a novel food, a new meta-analysis conducted by FSANZ evaluated the effects of CLA on LDL and HDL-cholesterol concentrations. The effects on blood concentrations of triglycerides were not assessed. The meta-analysis by FSANZ was different from one of the meta-analyses provided by the applicant (Clifton, 2009) in that it excluded studies that used a ruminant-derived mix containing *trans*-vaccenic acid and the *c-9,t-11* isomer of CLA (Desroches et al., 2005; Tricon et al., 2006), studies that used lactose as placebo (Yonei et al., 2007), and studies in which CLA was provided in milk or yoghurt drinks without replacing CLA with a fat as the placebo vehicle (Laso et al., 2007; López-Román et al., 2007). However, it included six additional studies (Aryaeian et al., 2009; Attar-Bashi et al., 2007; Herrmann, 2009; Park and Pariza, 2008; Sluijs et al., 2010; Zhao et al., 2009), some of which were unavailable at the time the Clifton (2009) meta-analysis was submitted.

Despite these differences in the selection of the studies and other differences regarding the methodology used for data analysis, the meta-analysis by FSANZ found a significant decrease in HDL-cholesterol concentrations which was half the size (2-3%) of that reported by Clifton (2009) and no significant changes in LDL-cholesterol concentrations during consumption of the 50:50 CLA mixture compared to control fats, some of which (e.g., *cis*-polyunsaturated fatty acids) are known to decrease LDL-cholesterol concentrations relative to isocaloric amounts of carbohydrates (Mensink et al., 2003).

The meta-analysis by Brouwer et al. (2010) provided in the ToR, that was designed to investigate the effects of animal and industrial *trans* fatty acids on LDL and HDL-cholesterol concentrations, evaluated 23 RCTs, 13 of which addressed the effects of CLA given as the 80:20 isomeric mixture of the *c-9, t-11* and *t10,c-12* isomers (n=3), as either isomer alone (n=1), or as the 50:50 isomeric mixture (n=9). The Panel notes that all these studies had already been considered in the

meta-analyses discussed above (Clifton, 2009; FSANZ, 2011). The Panel also notes that the potential sources of bias in the meta-analysis by Brouwer et al. (2010), already outlined in the opinion by ANSES (2011), do not allow conclusions to be drawn on the effects of CLA on blood lipids in humans.

The ANSES opinion (2011) also identified three recent reports on the effects of CLA on blood lipids that had not been included in the meta-analyses quoted above (Joseph et al., 2011; Pfeuffer et al., 2011; Venkatramanan et al., 2010). Two of them had a cross-over design, included 15 (Venkatramanan et al., 2010) and 36 (Joseph et al., 2011) overweight subjects, respectively, and provided CLA (as the *c-9,t-11* isomer or the 50:50 isomer mixture) at doses up to 2.8 g/day for 8 weeks. The third study had a parallel design, included about 20 overweight men per study arm, lasted 4 weeks and used the 1:1 CLA isomer mixture at a dose of 3.6 g/day (Pfeuffer et al., 2011). No effect of CLA on blood lipids was observed in any of these studies. Pfeuffer et al. (2011) also reported no effect of CLA on lipoprotein (a).

The Panel considers that the additional information provided does not contain evidence that would modify the previous conclusions reached by the Panel regarding the effects of CLA on blood lipids and lipoproteins.

3.3. Markers of lipid peroxidation and oxidative stress

The ANSES opinion (2011) identified four human intervention studies (Joseph et al., 2011; Pfeuffer et al., 2011; Smit et al., 2011; Turpeinen et al., 2008) on the effects of CLA on markers of lipid peroxidation that were not cited in previous EFSA opinions (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2010a, 2010b). Three of these reported on urinary excretion (Smit et al., 2011; Turpeinen et al., 2008) or plasma concentrations(Pfeuffer et al., 2011) of $F_{2\alpha}$ isoprostanes, whereas two reported on plasma concentrations of oxidised LDL (Joseph et al., 2011; Pfeuffer et al., 2011). One study (Kim et al., 2011) investigated the effects of CLA on other markers traditionally used to assess oxidative stress (TRAP, antioxidant enzymes, antioxidant vitamins). The Panel notes that changes in these markers do not indicate oxidative damage to molecules.

The study by Pfeuffer et al. (2011) had a parallel design, was conducted in overweight males, administered the 50:50 CLA isomeric mixture (3.6 g/day) for four weeks and used safflower oil as control. In the study by Turpeinen et al. (2008), 40 subjects with pollen allergy were randomised to consume 2 g CLA/day (an 80:20 mixture of the *c-9-t-11* and *t-10-c-12* isomers) or high-oleic acid sunflower-seed oil for 12 weeks. The study by Smit et al. (2011) was a cross-over study in which 61 adults described as healthy consumed three diets containing 7 % of the energy (about 20 g/day) as oleic acid (control), industrial *trans* fatty acids, or CLA (an 80:20 mixture of the *c-9-t-11* and *t-10-c-12* isomers) for three consecutive weeks each with no washout in between. Outcome variables were assessed at the end of each 3-week period. The Panel notes the limitations of this cross-over design and that CLA intakes were notably higher than those proposed for CLA as a novel food ingredient by the applicants.

All these studies reported a significant increase in urinary excretion (Smit et al., 2011; Turpeinen et al., 2008) or plasma concentrations (Pfeuffer et al., 2011) of 8-iso-$PGF_{2\alpha}$, as well as an increase in the urinary excretion of 15-*keto*-dihydro-$PGF_{2\alpha}$ (Turpeinen et al., 2008) with consumption of CLA compared to the control fat. No effect of CLA on plasma concentrations of oxidised LDL particles was reported in the two studies that addressed this outcome (Joseph et al., 2011; Pfeuffer et al., 2011).

An additional study (Kim et al., 2011) investigated the effects of CLA (1.8 g/day, 50:50 isomer mixture) given for eight weeks on the total antioxidant capacity of plasma and on plasma concentrations of antioxidant enzymes and antioxidant vitamins. No effect of CLA was reported on any of these variables relative to olive oil.

The Panel notes that the results of these studies are in line with the consistent increase in plasma and urinary concentrations of F2-isoprostanes reported in previous publications (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2010a, 2010b) and that, therefore, the additional information provided by these studies does not modify the conclusions reached by the Panel regarding the effects of CLA on lipid peroxidation.

3.4. Markers of systemic (subclinical) inflammation

The ANSES opinion (2011) identified nine human intervention studies on the effects of CLA on markers of subclinical systemic inflammation [high-sensitivity C-reactive protein (hs-CRP), tumor necrosis factor α (TNF-α), interleukin-6 (IL-6), IL-5, 15-*keto*-dihydro-$PGF_{2\alpha}$, prostaglandin E_2 (PGE_2), leukotriene B_4 (LTB_4), interferon-γ] or vascular inflammation [soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1), soluble E-selectin (sE-selectin)] not explicitly cited in previous EFSA opinions (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2010a, 2010b) in relation to this outcome (Aryaeian et al., 2009; Joseph et al., 2011; Mullen et al., 2007; Nugent et al., 2005; Pfeuffer et al., 2011; Sluijs et al., 2010; Smit et al., 2011; Turpeinen et al., 2008; Venkatramanan et al., 2010).

The studies by Nugent et al. (2005), Mullen et al. (2007), and Pfeuffer et al. (2011) had already been considered in the meta-analysis by Herrmann, (2009) provided by one of the applicants (Pfeuffer et al., 2009 as personal communication), whereas the studies by Aryaeian et al. (2009)and Turpeinen et al. (2008) were performed in subjects with ongoing inflammatory diseases (rheumatoid arthritis and pollen allergy, respectively) under anti-inflammatory medications and are difficult to interpret in relation to this outcome.

The remaining studies, which have been described in previous sections, did not report a significant effect of CLA on hs-CRP (Joseph et al., 2011; Sluijs et al., 2010; Smit et al., 2011; Venkatramanan et al., 2010), TNF-α (Joseph et al., 2011; Smit et al., 2011; Venkatramanan et al., 2010), IL-6 (Joseph et al., 2011; Smit et al., 2011), or markers of vascular inflammation (Smit et al., 2011).

In previous evaluations (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2010a, 2010b), the Panel noted that none of the studies evaluated had been designed to address the effects of CLA on subclinical inflammation, which also applies to the additional studies considered in this opinion with the exception of Smit et al. (2011), which used a cross-over design with significant limitations as outlined in section 3.3. Blood concentrations of 15-*keto*-dihydro-$PGF_{2\alpha}$ were consistently increased and blood concentrations of TNF-α and IL-6 were generally not affected by CLA treatment, which is in line with the additional, more recent studies available. The effects of CLA on hs-CRP had been inconsistent, with significant increases or no change in hs-CRP being reported in different studies of similar design. In a meta-analysis presented by one of the applicants (Herrmann, 2009), the 1:1 CLA mixture showed a significant increase in hs-CPR concentrations compared to the control group (six studies considered). Five of the studies were performed in subjects with a BMI >27 kg/m^2. The effects of CLA on markers of endothelial inflammation (namely ICAM-1, VCAM-1) could not be assessed due to the small number of studies available, from which the results were, moreover, inconsistent. The Panel notes that four new studies reported no effect of CLA on hs-CRP, that three of these studies were conducted in overweight subjects (Joseph et al., 2011; Sluijs et al., 2010; Venkatramanan et al., 2010) and that one of these had a bigger sample size than all the studies in the meta-analysis by Herrmann (2009) combined (n=173 per intervention group; Sluijs et al., 2010). However, the Panel also notes that the study by Sluijs et al. (2010) used 3.1 g/day CLA of the 80:20 *c-9, t-11* and *t-10,c-12* isomer mixture, in which the *t-10,c-12* isomer is underrepresented with respect to the conditions of use proposed by the applicants and to the blends used in the studies considered by Herrmann (2009). Only one new study reported on markers of vascular inflammation (Smit et al., 2011).

The Panel considers that the additional information provided does not contain evidence that would modify the previous conclusions reached by the Panel regarding the effects of CLA on markers of subclinical inflammation.

3.5. Vascular function and vascular damage

Two additional studies have been quoted in the ANSES opinion (2011) as new evidence with respect to previous EFSA opinions on the effects of CLA on vascular function (Pfeuffer et al., 2011; Sluijs et al., 2010). The results of one of these studies, which were provided to EFSA by the applicants in confidence, were already described in the EFSA opinions and referenced as Pfeuffer et al. (2009, unpublished).

The study by Sluijs et al. (2010) was conducted in 401 overweight or obese adults (342 analysed) aged 40–70 years who consumed either 3.1 g/day CLA (80:20 mixture of the *c-9-t-11* and *t-10-c-12* isomers) or a control fat (a blend of 80 % palm oil and 20 % soybean oil) in capsules for six months. No significant effect of the intervention was reported on arterial stiffness (measured as aortic pulse wave velocity), which was the primary outcome of the study, or on blood pressure.

In its previous opinions (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2010a, 2010b), the Panel considered the effects of CLA consumption on lipid peroxidation, subclinical inflammation and vascular function, their association with an increased risk of cardiovascular disease, and concluded that adverse effects on the arterial wall resulting from these changes, if any, would be expected to manifest only in the long term. The Panel notes that no additional information has become available on the effects of CLA intake on vascular function, vascular damage or atherosclerosis in humans for periods longer than six months that would modify the conclusions reached by the Panel regarding the potential effects of CLA on vascular damage.

3.6. Liver function and liver steatosis

Some animal studies had reported an increased lipid accumulation in the liver of mice fed high concentrations of CLA. The proposed mechanisms include (i) activation of peroxisome-proliferator-activated receptor (PPAR) regulated genes, (ii) increased plasma insulin and/or reduced leptin concentrations, and (iii) uptake of CLA into fat stores of the liver (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2010a, 2010b).

Most of the intervention studies with CLA conducted in humans already evaluated by the Panel did not report adverse effects on liver function enzymes at the proposed conditions of use. However, effects of CLA consumption over periods longer than six months on liver steatosis have not been adequately addressed in humans.

An additional study identified by the Panel (Wanders et al., 2010b) administered 19.3 g/day of the 80:20 CLA isomer mixture for 3 weeks to 20 healthy subjects with a BMI < 30kg/m^2 in replacement of other fatty acids in the usual diet with no clinically relevant effects on liver function enzymes. The Panel notes the very high dose of CLA used and the short duration of the study. The Panel also notes that the effects of CLA on liver function enzymes at the proposed conditions of use have already been found to be of no concern and that no studies are available on the effects of CLA on liver steatosis over periods longer than six months.

The Panel considers that no additional information has become available which would modify the previous conclusion of the Panel that the effects of CLA on liver steatosis have not been adequately addressed in human studies.

DISCUSSION

In its previous opinions (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2010a, 2010b), the Panel considered that CLA consumption did not appear to have adverse effects on insulin sensitivity, blood glucose control or liver function for periods up to six months, and that the observed effects on blood lipids were unlikely to have an impact on CVD risk. However, the observed increase in plasma and urinary concentrations of isoprostanes, which may indicate an increase in lipid peroxidation, and the increase in some markers of subclinical inflammation (i.e., 15-*keto*-dihydroprostaglandin $F_{2\alpha}$ and possibly CRP) associated with CLA consumption, together with the limited data available on the effects of CLA on vascular function, may indicate a potential for vascular damage (i.e., atherosclerosis) in the long term. Long-term effects of CLA intake on insulin sensitivity, the arterial wall or liver steatosis had not been adequately addressed in humans. The evidence provided did not establish the safety of CLA consumption by type-2 diabetic subjects under the proposed conditions of use.

The Panel considers that the additional information provided does not contain evidence that would modify the previous conclusions reached by the Panel regarding the effects of CLA on insulin sensitivity, blood glucose control, blood lipids, lipid peroxidation, or subclinical inflammation. The Panel also considers that the new studies provided do not address longer-term (> 6 months) effects of CLA intake on insulin sensitivity, the arterial wall or liver steatosis, or the safety of CLA in type-2 diabetic subjects, under the proposed conditions of use.

CONCLUSIONS

The Panel considers that the additional information provided does not contain evidence that would modify the previous conclusions reached by the Panel.

The Panel concludes that the safety of Clarinol® and Tonalin® TG 80, two oils with approximately 80 % of the CLA 50:50 mixture of *t-9,c-11* and *t-10,c-12* isomers, has been established for the proposed uses and daily doses (3.75 g Clarinol® and 4.5 g Tonalin® TG 80 corresponding to approximately 3 and 3.5 g of CLA, respectively) for up to six months. The safety of CLA consumption for periods longer than six months has not been established under the proposed conditions of use. The safety of CLA consumption by type-2 diabetic subjects has not been established.

DOCUMENTATION PROVIDED TO EFSA

Letter from the European Commission to the European Food Safety Authority with a request to review and update its opinions on the safety the "conjugated linoleic acid (CLA)-rich oils" Clarinol® and Tonalin® TG 80 as Novel Food ingredients.

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GLOSSARY AND ABBREVIATIONS

CLA	conjugated linoleic acid
hs-CRP	high-sensitivity C-reactive protein
HOMA-IR	homeostasis model insulin resistance index
PWV	pulse wave velocity
PPAR	peroxisome-proliferator-activated receptor
sVCAM-1	soluble vascular cell adhesion molecule-1
sICAM-1	soluble intercellular adhesion molecule-1
sE-selectin	soluble E-selectin
TRAP	plasma total radical-trapping antioxidant potential